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# Persistence Behaviour of Thiacloprid Residues in/on Green Tea Leaves, Processed Tea and Tea Infusion

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**Abstract** Thiacloprid residues were estimated in green tea leaves, processed tea and tea infusion by HPLC-Diode Array detection. The average initial deposits of thiacloprid (Alanto 240 SC) on the green tea leaves were found to be 3.72 and 6.77 μg $g^{-1}$ at single and double doses, respectively. The results showed that thiacloprid dissipated faster in green tea leaves following a first order reaction kinetics at both application rates. The amount of dissipation in 14 days was 93.37 % and 91.62 % for single and double doses respectively. Half life ($T_{1/2}$) for degradation of thiacloprid in green tea leaves were observed to be 3.34 and 3.58 days at single and double doses respectively. Thiacloprid residues in processed tea ranged from 0.16 to 0.63 μg $g^{-1}$ on seventh day and no residues could be detected on 14th day at single dose. Infusion study indicated that thiacloprid did not infuse into tea liquor from processed tea. The limit of determination was found to be 0.05 μg $g^{-1}$.

**Keywords** Thiacloprid · HPLC · Residues · Tea · Tea infusion

Tea (*Camellia sinensis L.*) is one of the most important cash crops in India due to its tremendous export potentialities and known as queen of beverages (Banerjee et al. 2010). It is the healthiest drink and second most consumed beverage after water (Biswas et al. 2007). India is the highest producer of tea in the world. Tea is attacked by several pests and diseases and it is imperative that plant protection chemicals are applied to reduce the severity of infestation. Among the factors limiting the quality and quantity of tea production, the role of insect pests is important. As a result, the tea planters use a wide range of pesticides to combat these problems for high yield and economic returns. Though broad-spectrum chemicals offer powerful incentives, they have serious drawbacks such as resistance to pesticides, pest resurgence, outbreak of secondary pests, harmful effects on human health and environment due to the presence of undesirable residues. Management of pests in tea fields largley depend on the use of conventional, neurotoxic, broad-spectrum, synthetic chemical pesticides, such as organophosphates, carbamates, synthetic pyrethroids and a number of new chemical classes, such as neonicotinoids.

Neonicotinoid insecticides represent the fastest growing class of insecticides introduced to the market since the launch of pyrethroids (Gupta and Shanker 2008). Thiacloprid (N-(3-(6-chloro-pyridin-3-ylmethyl)-thiazolidin-2-ylidene)-cyanamide) (Fig. 1) belongs to neonicotinoid group of insecticides (Gupta et al. 2008). Thiacloprid is a potent agonist of insect nicotinic acetylcholine receptors and disturbs synaptic signal transmissions (Tomlin 2006). It has a broad spectrum of activity against not only sucking insects but also chewing insects. Thus, there is a necessity for examining the residue and persistence of thiacloprid in tea crop for generating meaningful residue data from the supervised field trial of thiacloprid in tea so that it could be effectively utilized for the fixation of maximum residue limits (MRL) in our country. Therefore, a supervised field trial was conducted to determine the persistence behavior of thiacloprid residues in green tea leaves, processed tea and tea infusion.

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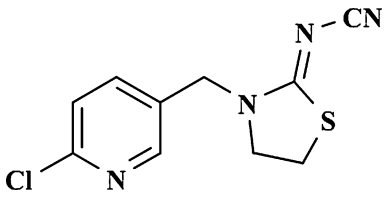


**Fig. 1** Chemical structure of thiacloprid

## Materials and Methods

Pesticide analytical standard of thiacloprid (99.6 % purity) and commercial formulation (Alanto 240 SC) was supplied by Bayer CropScience, India. Stock solution(1000 μg $mL^{-1}$) of thiacloprid was prepared in acetonitrile. Calibration solutions were prepared with different concentration just before use. The standard solutions were kept under refrigerated condition, protected from light. All the solvents and chemicals used in this study were of analytical grade from Merck, Mumbai (India).

The field experiment was conducted in a randomized block design (RBD) replicated thrice at Coonoor Tea Estate, Tamil Nadu, India. Plots measuring 100 sq m, containing tea plants of TRF-1 cultivar with appropriate guard rows, were used for the study. Tea plants had been planted in double hedge, in triangular planting system at spacing of 30 × 45 cm. The commercial formulation of thiacloprid 240 SC (Alanto) was applied to tea bushes twice @ 90 g a.i./ha (recommended dose) and 180 g a.i./ha (double the recommended dose) by a Knapsack sprayer and along with untreated control. The volume of water used was 400 L/ha.

Green tea leaves (Two leaf and a bud, 2 kg) were plucked randomly from each treatment replication wise at time 0 (2 h after application), 3, 5, 7, 10 and 14 days after the application of thiacloprid. The green tea leaf samples (2 kg) of 7 and 14 days were then processed in the tea garden at Coonoor Tea Estate factory following standard manufacturing methods to get processed tea (100 g). The green tea samples were brought to the laboratory packed in dry ice from the field and processed immediately for residue analysis. Soil samples (1 kg) cropped with tea was collected at harvest.

The homogenized green tea leaves (2 g) and processed tea (10 g) samples were extracted with 150 and 50 mL of acetone:water (3:1, v/v) respectively, by keeping it in a mechanical shaker for 2 h at 250 rpm. The contents were filtered through Celite 545 (5 g) filter aid using Whatman 42 filter paper supported on a Buchner funnel. The filtered solids were again washed with another 70 mL of acetone/water (3/1, v/v) and filtered solids were discarded. The filtrate was evaporated to an aqueous remainder of 20 mL using a rotary vacuum evaporator with a bath temperature of 40 °C.

The aqueous solution was placed on the top of the ChemElut (20 mL volume) column and allowed to stand for 15 min to achieve an equal distribution of the liquid on the column. Thiacloprid residues were eluted from the column with 60 mL of cyclohexane/ethyl acetate (1/1, v/v). The eluent was concentrated to dryness using a rotary vacuum evaporator with a bath temperature of 40 °C. The residue was reconstituted in 2 mL of ethyl acetate and cleaned up by adsorption column chromatography using 10 g of 5 % deactivated florisil. The column was washed with 50 mL of ethyl acetate and eluate was discarded. Thiacloprid residues were eluted with 100 mL of acetonitrile and the eluate was evaporated to dryness using a rotary vacuum evaporator at a temperature of 40 °C. The residues were dissolved in 1 mL of acetonitrile/water (25/75, v/v) and HPLC analysis was carried out with an injection volume of 20 μL.

Processed tea (5.0 g) was boiled in 100 ml of distilled water to get the tea liquor. After 6 min of brewing, the water extract was filtered through Celite 545 (5 g) filter aid using Whatman 42 filter paper supported on a Buchner funnel. The samples were extracted with cyclohexane: ethyl acetate 1:1 (v/v) (3 × 60 mL) by adding 10 mL saturated sodium chloride. The organic phase was passed through anhydrous sodium sulphate. The extract was concentrated by evaporating in a rotary vacuum evaporator and diluted with 2 mL of ethyl acetate. The cleanup procedure was followed as green tea leaves method.

Soil sample was air dried and sieved for each replication/treatment. About 10 g of the soil sample was taken in a centrifuge tube and 20 mL of ethyl acetate was added and was shaken well. Then anhydrous $MgSO_4$ (4 g) and NaCl (1 g) was added to this and shaken well. This solution was centrifuged at 10,000 rpm for 10 min. About 8 mL of the supernatant solution was taken from this and 100 mg of PSA and 600 mg of $MgSO_4$ was added and shaken well. This was centrifuged at 5,000 rpm for 10 min and 4 mL of the supernatant was taken from this into a Turbovap tube and was concentrated to dryness. Then its volume was made up with 1 mL of acetonitrile/water (25/75, v/v) and HPLC analysis was carried out with an injection volume of 20 μL.

In order to evaluate the extraction efficiency and reliability of the analytical method adopted, recovery study was carried out by fortifying green tea leaves, processed tea, tea infusion and soil at the level of 0.05 μg $g^{-1}$ (LOQ level), 0.25 μg $g^{-1}$ (5 times LOQ level) and 0.50 μg $g^{-1}$ (10 times LOQ level) for irrespective of substrates, separately with analytical standard of thiacloprid. The fortified sample was left standing for 30 min to allow even distribution of the pesticide and give time to interact with the matrix. The samples were processed by adopting above procedure.

**Table 1** Recoveries of thiacloprid on green tea leaves, processed tea, tea infusion and soil

| Fortified concentration (μg $g^{-1}$) | Recovery percentage* ± SD | | | |
|---|---|---|---|---|
| | Green tea leaves | Processed tea | Tea infusion | Soil |
| 0.05 | 91.02 ± 2.03 | 88.71 ± 2.76 | 92.73 ± 2.45 | 96.39 ± 4.59 |
| 0.25 | 86.68 ± 4.61 | 85.52 ± 6.32 | 96.46 ± 2.47 | 96.38 ± 2.04 |
| 0.50 | 110.15 ± 4.81 | 96.18 ± 3.93 | 93.18 ± 1.29 | 89.48 ± 8.61 |

* Average of three replicates, *SD* standard deviation

**Table 2** Residues of thiacloprid on green tea leaves

| Days after treatment | Residues recovered* (μg $g^{-1}$) ± SD | | | | |
|---|---|---|---|---|---|
| | Control | 90 g a.i/ha | Dissipation (%) | 180 g a.i/ha | Dissipation (%) |
| 0 (2 h after spray) | ND | 3.726 ± 0.212 | 0.00 | 6.777 ± 0.024 | 0.00 |
| 3 | ND | 2.677 ± 0.042 | 21.73 | 4.948 ± 0.217 | 23.42 |
| 5 | ND | 1.156 ± 0.055 | 66.20 | 1.921 ± 0.137 | 70.66 |
| 7 | ND | 0.821 ± 0.068 | 76.00 | 1.375 ± 0.117 | 78.99 |
| 10 | ND | 0.478 ± 0.044 | 86.04 | 0.754 ± 0.058 | 88.48 |
| 14 | ND | 0.227 ± 0.019 | 93.37 | 0.548 ± 0.053 | 91.62 |
| Regression equation | – | Y = 3.587−0.090x | | Y = 3.810−0.084x | |
| Half-life (days) | – | 3.34 | | 3.58 | |

* Average of three replicates, *ND* not detected, *SD* standard deviation

The residues of thiacloprid were determined using Shimadzu HPLC (Shimadzu—LC 20AT) equipped with diode array detector (SPD-M20A) and online degasser (DGU-20As). A reversed-phase Chromolith® Performance, C18 column 10 cm long, 4.6 mm i.d) at ambient temperature and Labsolution software system was employed to acquire and process chromatographic data throughout the experiment. Acetonitrile:water (25:75,v/v) was used as mobile phase for the detection of thiacloprid residue. The program was set for an isocratic elution with flow rate of 1 mL $min^{-1}$, wavelength, 245 nm and the aliquots of 20 μL of the samples were injected. All the solvents were filtered with a 0.45 mm membrane filter. With these operating parameters the retention time of thiacloprid was at 5.09 min.

## Results and Discussion

The described analytical method was optimised for the analysis of thiacloprid residues in green tea leaves, processed tea, tea infusion and soil samples by HPLC. Quantification was accomplished by using a standard curve, prepared by diluting the stock solution in acetonitrile/water (25:75 v/v). Good linearity was achieved with a correlation coefficient of 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were determined as 0.01 and 0.05 μg $g^{-1}$, respectively. No control samples showed any evidence of chromatographic interference.

The extraction efficiency of the method has been evaluated by spiking green tea leaves, processed tea, tea infusion and soil samples with thiacloprid working solutions at various levels (0.05 μg $g^{-1}$ -(LOQ level), 0.25 μg $g^{-1}$ (5 times LOQ level) and 0.50 μg $g^{-1}$ (10 times LOQ level). In green tea leaves recovery of thiacloprid ranged between 86.68 % and 110.15 %, whereas, in processed tea and tea infusion it was 85.52 to 96.18 % and 92.73 to 96.46 % respectively (Table 1). The recovery percentage in soil ranged between 89 and 96 %. All of these values of recovery indicated good method accuracy and repeatability, and are within the accepted range for residue determinations.

The results of thiacloprid residue analysis and the percent dissipation at different intervals at single and double doses were presented in Table 2. No residues of thiacloprid was detected in any analysed control tea sample. The initial mean deposit of thiacloprid in green tea leaves were 3.72 and 6.77 μg $g^{-1}$ for 90 and 180 g a.i $ha^{-1}$ respectively. In both the concentrations the level of thiacloprid resdiues dissiapted on fifth day was more than 64 per cent i.e., 66.20 and 70.66 per cent at recommended dose and double of the recommended dose, respectively (Table 2). On fourteenth day, the thiacloprid residues dissipated faster and reached above 90 percent i.e., 93.37 and 91.62 in green tea leaves at

**Table 3** Residues of thiacloprid on processed tea, tea infusion and soil

| Substrate | Days after treatment | Residues recovered* (μg $g^{-1}$) ± SD | | |
|---|---|---|---|---|
| | | Control | 90 g a.i/ha | 180 g a.i/ha |
| Processed tea | 7 | ND | 0.167 ± 0.004 | 0.633 ± 0.099 |
| | 14 | ND | BDL | 0.362 ± 0.022 |
| Tea infusion | 7 | ND | BDL | BDL |
| | 14 | ND | BDL | BDL |
| Soil | 14 | ND | BDL | BDL |

* Average of three replicates, *BDL* Below detectable level (<0.05 μg $g^{-1}$), *ND* not detected

both the doses. The residues dissipated at the half-life of 3.34 and 3.58 days from the two treatments (Table 2) and suggested that the dissipation was dependent on initial dose of thiacloprid and followed a first order reaction kinetics.

On seventh day the average residue level of thiacloprid in processed tea was 0.167 and 0.633 μg $g^{-1}$ at single and double dose treated plots. On recommended dose the residue was below determination level at 14th day and incase of double the recommended dose the residue was 0.362 μg $g^{-1}$ (Table 3). The residue levels of thiacloprid in tea infusion on 7 and 14th day were below detectable level (BDL) at 90 and 180 g a.i $ha^{-1}$ doses (Table 3). Soil samples collected at harvest had no detectable residues of thiacloprid.

From this result, it revealed that no residue of thiacloprid was detected in processed tea on 14 days after application, at the recommended dose application dose (90 g a.i/ha). Thus, this insecticide can be safely recommended for any pest management programme in tea plantations.

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